IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No.:	10/564,166	Group Art Unit:	1626
Filed:	August 7, 2006	Confirmation No.:	7842
Applicants:	Berdini *et al.*	Examiner:	Stockton, Laura
Title:	BENZIMIDAZOLE DERIVATIVES AND THEIR USE AS PROTEIN KINASES INHIBITORS		

DECLARATION UNDER 37 C.F.R. §1.132

To: Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, David Charles Rees, declare:

1. I am employed as the Senior Vice President, Medicinal Chemistry at Astex Therapeutics Limited, located at 436 Cambridge Science Park, Milton Road, Cambridge CB4 0QA, United Kingdom, the assignee of the above-identified application (hereinafter "the Assignee"). I have been employed at the Assignee since January 2003.

2. I have a B.Sc. and Ph.D. in Chemistry. I am a co-recipient of the Royal Society of Chemistry Malcolm Campbell Memorial Prize 2007 for the discovery of the anaesthesia drug sugammadex whilst at Organon. Sugammadex (Bridion®) received regulatory approval in Europe in July 2008. Prior to Astex, I have 19 years experience as a medicinal chemist in the pharmaceutical industry, working with Parke-Davis, Organon and, most recently, AstraZeneca where I held the position of Director and Head of the Medicinal Chemistry Department with some 140 staff at the research and development laboratories in Mölndal, Sweden. I am co-author of over 75 patent applications and publications, including Howard *et al.* referred to in Paragraph 7 below. I have served as an honorary Professor at Glasgow University and as a member of the Royal Society of Chemistry Organic Division Executive.

3. I am authorized to make this declaration on behalf of the Assignee.

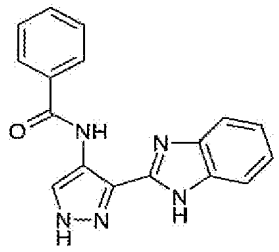
4. I am well acquainted with the facts stated herein as a consequence of my personal knowledge and from records maintained by the Assignee.

5. U.S. patent application number 10/564,166 (hereinafter referred to as "the Patent Application"), which is a 35 U.S.C. § 371 national stage application of International patent application number PCT/GB2004/02824, describes a class of substituted pyrazolylbenzoimidazoles that have activity as inhibitors of various kinases and in particular cyclin dependent kinases (CDK), glycogen synthase kinase-3 (GSK-3) and Aurora kinases.

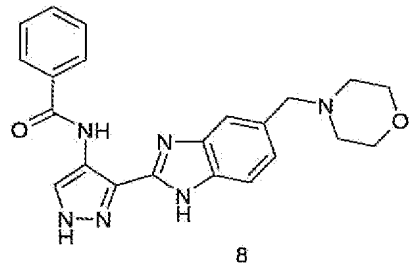
6. Experiments carried out in the Assignee's laboratories have demonstrated that compounds having a morpholinylmethyl substituent group on the benzene ring of the benzoimidazole group (and corresponding to formulae (VII) and (VIIa) of the Patent Application as originally filed) have good activity against CDK, GSK-3 and Aurora kinases. More particularly, such compounds have been found to have increased affinity for Aurora kinases and in particular Aurora kinase B.

7. The advantages conferred by the presence of the morpholinylmethyl group are discussed in the enclosed paper by Howard *et al.*, *J. Med. Chem.*, 2009, 52, 379-388 (hereinafter referred to as "Exhibit 1"), a copy of which is attached hereto and incorporated herein as Exhibit 1.

8. The effect of the morpholinylmethyl group is illustrated in Table 1 on page 380 of Exhibit 1 where there is a comparison between compounds having an unsubstituted benzoimidazole benzene ring and compounds in which the benzene ring of the benzoimidazole is substituted by a morpholinylmethyl group. The data in Table 1 allow a comparison to be made between the compounds 7 and 8 shown below.



7



8

9. The inhibitory activities against the kinases Aurora A, Aurora B, CDK1/B and CDK2/A and the human colon carcinoma cell line HCT116, as shown in Table 1 of Exhibit 1, are set out in Table A below.

Table A (from Table 1 of Exhibit 1)

No.	IC_{50} (μM)*				HCT116 assay (μM)*
	Aurora A	Aurora B	CDK1/B	CDK2/A	
7	0.0059	0.18	0.23	0.052	3
8	0.0035	0.015	0.34	0.14	0.1

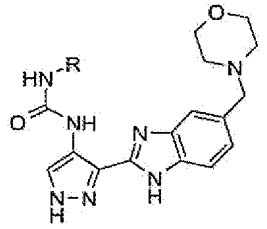
*as determined using the methods described in Howard *et al.*, *J. Med. Chem.*, 2009, 52, 379-388

10. The data demonstrate that the introduction of the morpholinylmethyl group leads to an approximately ***10 fold increase*** in activity against Aurora B and a modest improvement in activity against Aurora A.

11. The data also show that the introduction of the morpholinylmethyl group leads to a ***30 fold increase*** in activity against the human colon carcinoma cell line HCT116.

12. The potent activity of the compounds is also demonstrated by the data in Table 2 on page 382 of Exhibit 1. The data show that all of the compounds disclosed in Table 2 have better activity against the human colon carcinoma cell line than compound 7.

Table 2 (from Exhibit 1)



No.	R	IC_{50}(μM)*				HCT116 assay (μM)*
		Aurora A	Aurora B	CDK1/B	CDK2/A	
10	phenyl	0.012	0.054	5.5	1.8	0.3 – 1
11	2-fluorophenyl	0.0028	0.010	1.1	0.23	0.1-0.3
12	2,6-difluorophenyl	0.0015	0.0026	0.29	0.16	0.03
13	cyclohexyl	0.0057	0.0052	2.9	1.2	0.1
14	3-pyridinyl	0.013	0.024	0.29	0.16	1-3
15	4-tetahydropyranyl	0.026	0.010	17	NT	1
16	cyclopropyl	52% I @ 3 nM	58% I @ 3 nM	1.7	0.51	0.03

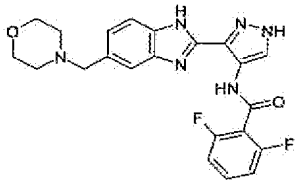
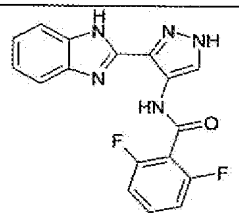
*as determined using the methods described in Howard *et al.*, *J. Med. Chem.*, 2009, 52, 379-388

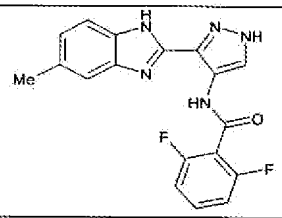
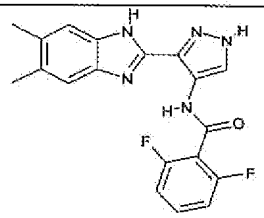
13. The data in Table 2 of Exhibit 1 illustrate that the enhanced activity of compounds containing a morpholinylmethyl substituent is retained over a wide range of different types of substituent R^{1d} in formulae (VII) and (VIIa) in the Patent Application. In particular, the data show that excellent activity is retained in compounds where R^{1d} is aromatic (compounds 10, 11 and 12), cycloalkyl (compounds 13 and 16), heteroaromatic (compound 14) and saturated heterocyclic (compound 15).

14. In Example 306 in the Patent Application, assays for determining the activity of the compounds as inhibitors of CDK2 are described and it is stated that the compounds of Examples 3 to 128 each have IC_{50} values of less than 20μM or provide at least 50% inhibition of the CDK2 activity at a concentration of 10μM. In Examples 307, 311 and 312 of the Patent Application, assays are described for testing the activities of the compounds as inhibitors of Aurora A kinase, GSK3-β kinase and the human colon carcinoma cell line HCT116.

15. By using the protocol described in Example 311 of the Patent Application, or methods differing therefrom in only minor respects, the activities of the compounds of Examples 6, 22, 73, and 84 of the Patent Application against human colon carcinoma cell line HCT116 were determined. The results are shown in Table B below.

Table B

Patent Example		**HCT-116 (prolif)(μM)** [§]
22		0.027
6		0.12

Patent Example		HCT-116 (prolif)(μM) [§]
84		0.25
73		0.49

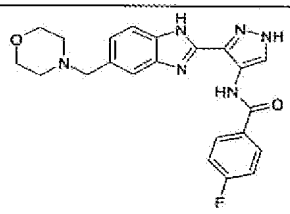
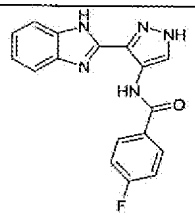
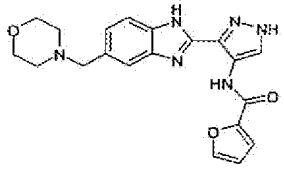
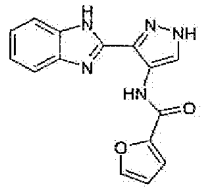
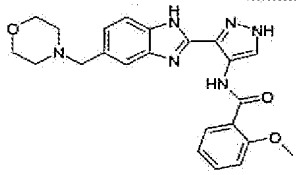
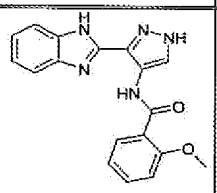
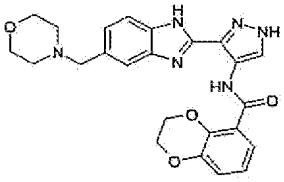
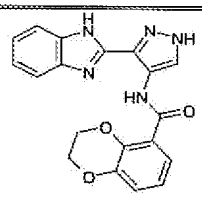
[§]where more than one assay experiment performed number shown is the arithmetic mean of the results obtained

16. In each of the examples above, the compounds have the same 2,6-difluorobenzoylamino group attached to the 4-position of the pyrazole ring but have different or no substituent groups attached to the benzoimizadole ring. The data therefore allow a direct comparison to be made between the activities of compounds of formulae (VII) and (VIIa) having a morpholinylmethyl side chain on the benzoimidazole group and compounds having no substituents or alternative substituents on the benzoimidazole ring. Compound 22 has the morpholinylmethyl side chain required by the formulae (VII) and (VIIa) whereas the compounds of Examples 6, 73 and 84 all contain benzoimidazole motifs that lack a morpholinylmethyl side chain.

17. The cell assay data illustrate that the compound bearing the morpholinylmethyl side chain (Example 22) is approximately ***four times as active*** as the compound of Example 6, is approximately ***nine times as active*** as the compound of Example 84, and is approximately ***eighteen times as active*** as the compound of Example 73.

18. This improvement in cellular activity arising from the presence of a morpholinylmethyl side chain is further evidenced by the following pairs of compounds in Table C below whereby the morpholinylmethyl-substituted compounds are shown to have significantly better activity than the corresponding unsubstituted benzoimidazole compounds shown on the right hand side of the table.

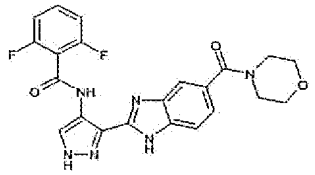
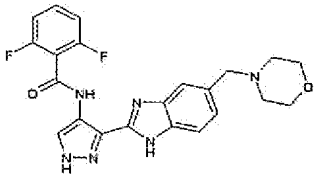
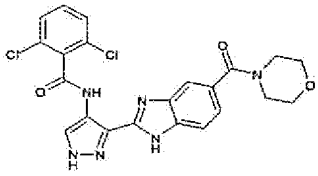
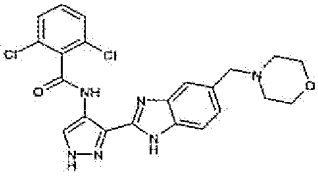
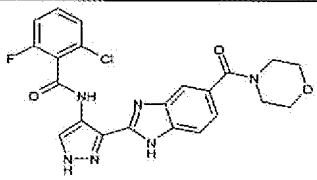
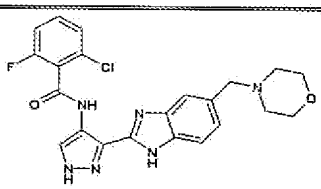
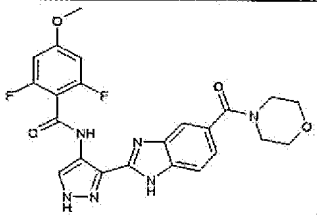
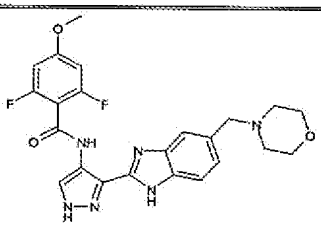
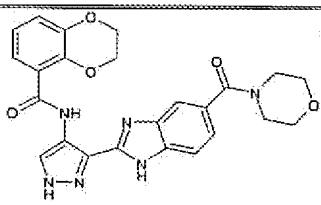
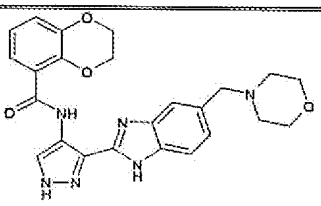
Table C

Patent Example		HCT-116 (prolif) (μM) [§]	Patent Example		HCT-116 (prolif) (μM) [§]
223		0.25 μM	n/a		73% at 10 μM
219		0.036	39		0.71
164		0.033	38		0.36
97B		0.054	36		0.22

[§]where more than one assay experiment performed number shown is the arithmetic mean of the results obtained

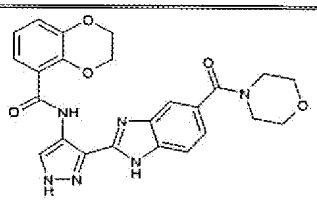
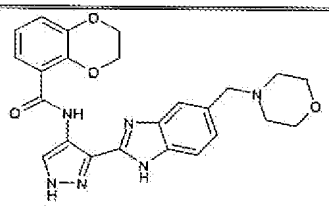
19. Table D compares compounds bearing a morpholinylmethyl side chain, which exhibit basic properties, with compounds bearing a morpholinylamide side chain which, in contrast, do not. The cell assay data show that the compounds bearing the morpholinylmethyl side chain (Examples 22, 94E, 95B, 96B, 97B) are, in each case, more active than those bearing the morpholinylamide side chain (Examples 68, 98-101). This is consistent with Aurora kinase activity (Table E) where the morpholinylmethyl analogue (Example 97B) is ***eighty times as*** active as the morpholinylamide analogue (Example 101).

Table D

Patent Example		HCT-116 (prolif) (μM) [§]	Patent Example		HCT-116 (prolif) (μM) [§]
68		0.13	22		0.026
98		0.21	94E		0.08
99		0.15	95B		0.04
100		0.57	96B		0.23
101		0.51	97B		0.048

[§]where more than one assay experiment performed number shown is the geometric mean of the results obtained

Table E

Patent Example		Aurora A (μM) †§	Patent Example		Aurora A (μM) †§
101		0.77	97B		0.0095

§where more than one assay experiment performed number shown is the geometric mean of the results obtained

†data generated using similar methods to those described in Example 307

20. The data presented in the application, Exhibit 1, and the tables above clearly demonstrate that the presence of a morpholinylmethyl side chain results in substantially improved activity against Aurora kinase and improved efficacy in a cellular assay compared to compounds in which the morpholinylmethyl group is absent.

21. I further declare that all statements of the foregoing declaration made of my own knowledge are true and that all statements made upon information and belief are believed true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above identified application or any patent issuing thereon.

Signed by me this 16th day of September, 2010.

DAVID CHARLES REES